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13. ABSTRACT (Maximum 200 Words)
Studies in Year 2 continued according to the original plan. Studies were carried out in RBE4 cells and in primary astrocyte cultures. In both cell types, there was a significant incrase in ^{54}manganese uptake due to the addition of uranium to the media. Incubation with uranium did not significantly alter DMT-1 may not be the putative transporter for uranium across the blood-brain barrier. In addition studies, which were delayed by the lack of availability of DU pellets, we implanted rats in the gastrocnemius muscle with 10 pellets in each leg for a total of 20 pellets. The following doses were administered: sham=20 tantalum (Ta) pellets; **low**=16 Ta and 4 du; **medium**=10 Ta and 10 du; **high**=4 Ta and 16 du. The three-month cohort will be analyzed shortly for uranium brain levels, as well as DMT-1 expression in various brain regions. Rats have been monitored (e.g., body weights, coat quality), and there are no noted differences in body weights in either the three-month or six-month cohorts (Figure 4) or physical appearance have occurred in rats receiving surgery (both Ta and du pellets) compared to non-surgical controls.

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INTRODUCTION

(Based on original abstract)

Recent studies of Gulf War veterans with depleted uranium (DU) embedded fragments in their soft tissues point to DU-induced effects on neurobehavioral and cognitive function (McDiarmid et al., 2000). These observations are corroborated by electrophysiological changes in hippocampal slices isolated from rats embedded with DU fragments (Pellmar et al., 1999a; Pellmar et al., 1999b). Notably, studies from the same group also suggest, for the first time, that uranium accumulates within brain tissue (Pellmar et al., 1999a). It is presently unknown how uranium is transported into the brain, and there are no pharmacological modalities to reduce its accumulation within the central nervous system (CNS). The purpose of this project is to identify the substrate specificity of uranium transport in the CNS, the working hypothesis being that the divalent metal transporter (DMT-1) which has an unusually broad substrate range that includes Fe^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, and Pb^{2+}, is mediating uranium transport in the CNS. This project focuses on examining this hypothesis from an in vitro approach utilizing endothelial cell culture models (Technical Objective 1.0) as well as an in vivo approach to delineate the pharmacokinetics of uranium transport across the BBB in rats embedded with DU fragments (Technical Objective 2.0). The studies will test the hypothesis that a relationship exists between blood and brain uranium concentrations, determining whether rats with the highest blood uranium concentrations also accumulate the highest uranium concentrations in the CNS. Thus, the studies will facilitate risk assessment in veterans, and will determine whether those with the highest uranium blood levels are more prone to accumulate uranium in the CNS compared to veterans with low blood uranium levels.

BODY

Stated Technical Objectives for Year 2 of this proposal were:

1.0 To determine the in vitro transport of uranium across the BBB in *in vitro* endothelial cell culture models (RBE4 and bovine brain endothelial cells).

2.0 Study the *in vivo* transport of uranium across the BBB.

Technical Objective 1:

During the second year of the project, studies were performed in which uranium transport was assessed both directly and indirectly utilizing the in vitro cell culture model system.
Earlier we showed that the divalent metal transporter (DMT-1) is present in the RBE4 cells and that treatment with desferroxamine (DFO; an iron chelator) causes a significant increase in DMT-1 levels in these cells (Lack et al., 2003). Since we hypothesize that DMT-1 is a putative mediator of uranium transport into the brain, we tested the direct effect of uranium on DMT-1 protein levels in addition to the interactions of uranium with manganese transport. This latter experiment was based on the fact that manganese is known to be transported in the brain via DMT-1 (Conrad et al., 2000,

Erikson et al., 2003). We postulated that if cells are incubated with varying concentrations of uranium, then manganese transport should be attenuated due to the competition between the two metals for cellular transport. These studies were carried out in RBE cells and in primary astrocyte cultures. We found that in both cell types, there was a significant increase in ^{54}manganese uptake due to the addition of uranium to the media (Figure 1). To directly examine the affect of uranium on DMT-1 levels, experiments were conducted in which RBE4 cells were incubated with uranium for 24 hours and DMT-1 levels were measured via western blot analysis.

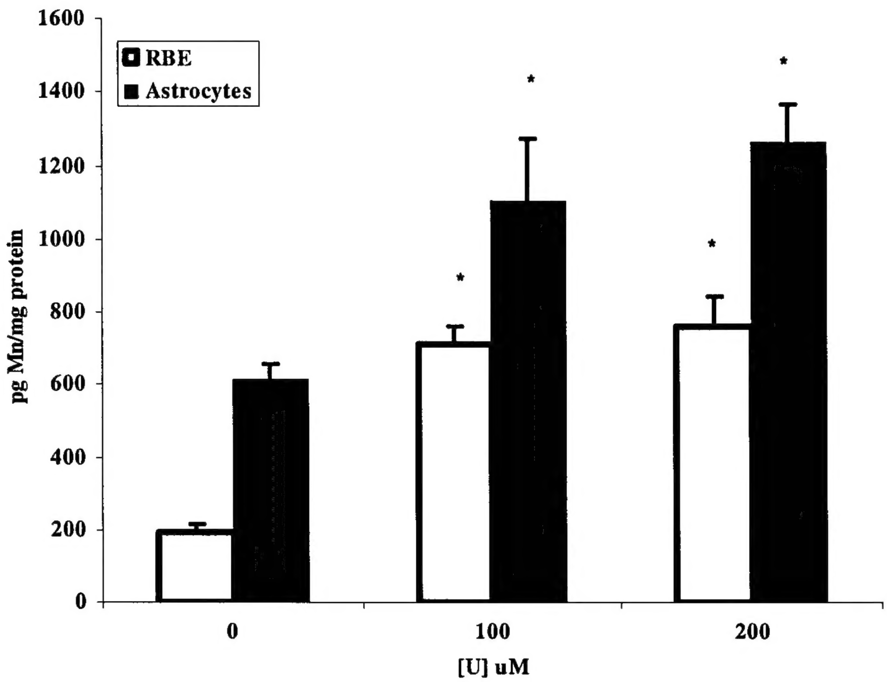

Figure 1: Immortalized Rat Brain Endothelial 4 (RBE4) cells and primary astrocyte cultures were incubated with 0, 100 or 200 μM uranium for 24 hours. ^{54}Mn uptake studies performed the following day indicated that uranium caused a significant increase in manganese, but that this effect was not dose dependent. Data are mean ± SEM from 8 plates/4 culture dates or n=32 plates for RBE4 cells; 8 plates/ 3 culture dates or n=24 plates for primary rat astrocytes.

Incubation with uranium did not significantly alter DMT-1 levels when compared to control cells (Figure 2). Collectively, both experiments do not definitively support nor refute the role of DMT-1 in uranium transport in the CNS. Currently, RBE4 cells overexpressing DMT-1 are being prepared for uranium transport studies.

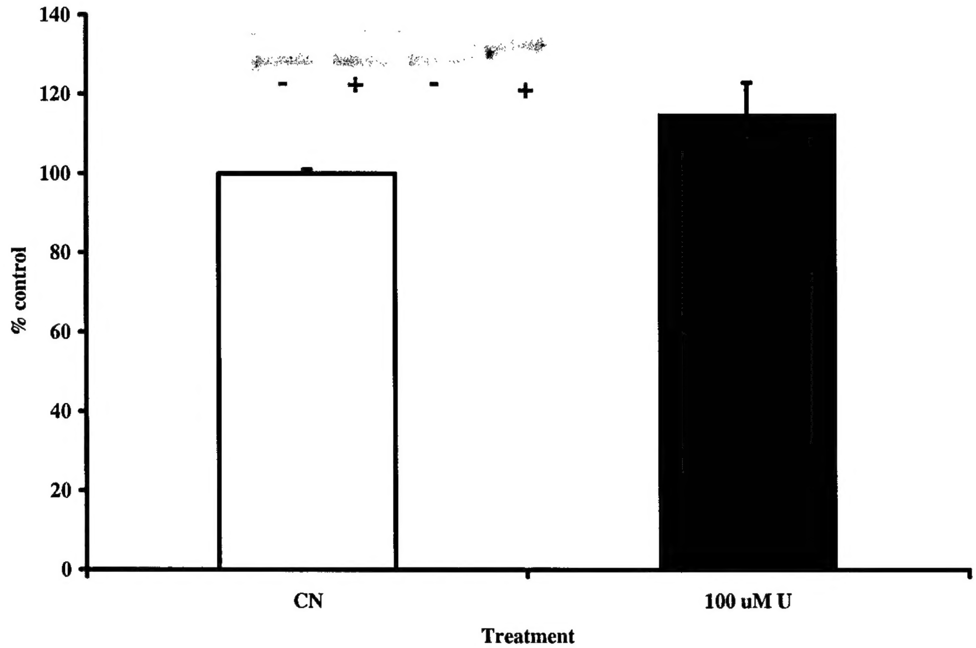

Figure 2: Western Blot Analysis of DMT-1 in RBE4 cells treated in the presence of 100 uM U (+) or absence (-) for 24 hours. There was a non-significant increase in DMT-1 levels after uranium treatment. Data are mean ± SEM of 3 plates/2 culture dates or n=6 plates).

Technical Objective 2:

The focus of this second year has been on performing the in vivo studies that were proposed. Depleted uranium (du) pellets arrived June 2003 (they were ordered in January 2003) and we began surgically implanting them in rats for in vivo studies. Briefly, rats were implanted with 10 pellets in the gastrocnemius muscle of each leg for a total of 20 pellets. The following doses were administered: **sham**=20 tantalum (Ta) pellets; **low**=16 Ta and 4 du; **medium**=10 Ta and 10 du; **high**=4 Ta and 16 du. Seven rats per group were implanted with pellets in addition to a non surgical control group which is being maintained for each group for a total of 35 rats per time period. We performed this procedure on rats representing two different time points, the six month and three month cohorts.

The surgical procedure was as follows:

Rats were anesthetised with a ketamine (80 mg/kg) and xylazine (12 mg/kg) mixture. The rats were then shaved completely from tail to abdomen and the area cleaned with betadine followed by alcohol. The rats were then placed in an aseptic field via a sterilized drape. A 3.0 cm incision using a #10 scalpel blade parallel to the leg was made to expose the gastrocnemius muscle. Fascia was teased away. An 18 gauge needle was then used to create a hole in the muscle and then a pellet was placed using a 16 gauge needle and stylet. This procedure was repeated every 1.5 mm until 10 pellets were placed. The wound was then closed using a 4-0 Ethilon non-absorbable nylon suture

with a PS-2 cutting needle. The procedure was then repeated on the opposite leg. Triple antibiotic ointment was applied to the wound and the animal was allowed to recover overnight under a light.

Rats have been monitored (e.g., body weights, coat quality) twice weekly since surgery. Since August 4, 2003 when official monitoring began, no noted differences in body weights in either the three-month (Figure 3) or six-month cohorts (Figure 4) or physical appearance have occurred in rats receiving surgery (both Ta and du pellets) compared to non-surgical controls. The three-month exposure group is scheduled to be killed the week of October 27, 2003 and the six-month group the week of January 5, 2004. Briefly, the rats will be perfused with phosphate-buffered saline to ensure all blood is removed from the animals. This is an essential component to this project, because the couple of studies that reported increased U in brains of implanted rats did not perfuse the rats, thereby leaving it unclear as to whether the increase U is associated with brain tissue or blood. Brains, livers, kidneys, hearts and other tissues will be harvested; brains will be dissected into seven brain regions: caudate putamen, cerebellum, cortex, globus pallidus, hippocampus, substantia nigra, and thalamus. Brain regions and other tissues will be frozen at -80° C until analysis.

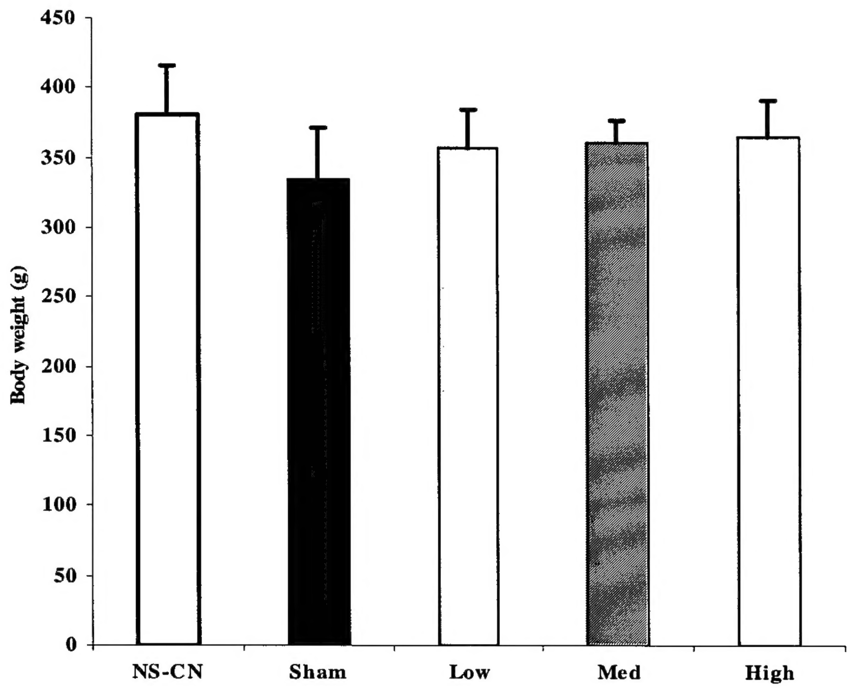

Figure 3: Body weights of rats surgically implanted with depleted uranium pellets from the three-month cohort (n=7/group). Non-surgical Control (NS-CN); sham=20 tantalum (Ta) pellets; low=16 Ta and 4 depleted uranium (du); medium=10 Ta and 10 du; high=4 Ta and 16 du. There is no statistical difference in body weights of the rats from the three-month cohort when comparing the non-surgical controls to each of the other groups. Data are reported as mean ± standard deviation.

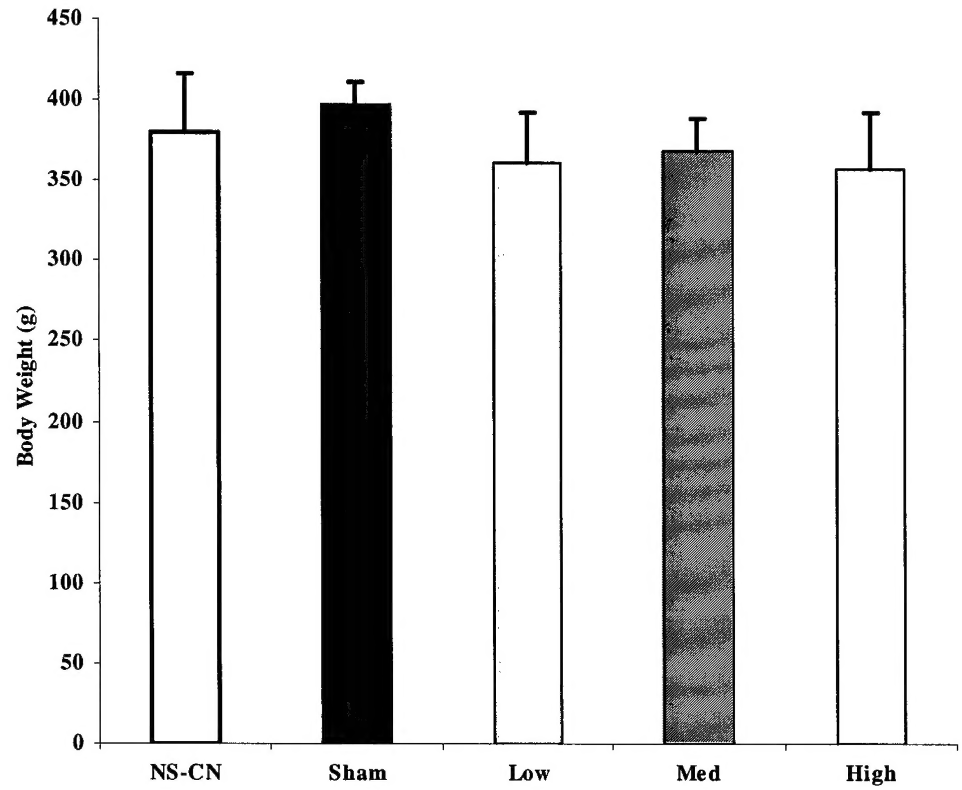

Figure 4: Body weights of rats surgically implanted with depleted uranium pellets from the six-month cohort (n=7/group). Non-surgical Control (NS-CN); sham=20 tantalum (Ta) pellets; low=16 Ta and 4 depleted uranium (du); medium=10 Ta and 10 du; high=4 Ta and 16 du. There is no statistical difference in body weights of the rats from the three-month cohort when comparing the non-surgical controls to each of the other groups. Data are reported as mean ± standard deviation.

Uranium concentrations will be measured utilizing inductively coupled plasma mass spectrometry (ICP-MS). We will then statistically determine if any brain region(s) accumulated U significantly. This assessment will direct future studies in terms of repeating the procedure for shorter durations (e.g., 7, 14 or 30 day exposures) as well as pharmacokinetic-type studies. For example, if there is no brain regional accumulation of uranium after three months of exposure, than shorter term exposures may not be necessary. Likewise, by grasping which brain regions readily accumulate U, in vivo microdialysis studies (pharmacokinetic studies) can be precisely planned to include the most vulnerable brain region(s).

KEY RESEARCH ACCOMPLISHMENTS

- Western Blot analysis of DMT-1 levels in RBE cells treated with Uranium
- 54-Mn uptake studies in RBE and astrocyte cultures incubated with Uranium to examine interactions between U and Mn transport.
- Received depleted uranium pellets from Aerojet Ordinance in June 2003, implanted 3 month and 6 month cohorts of rats sham, low, medium, high dose groups (n=7).
- Surgeries completed week of July 28, 2003. Scheduled to kill 3 month group week of October 27, 2003 and 6 month group week of January 5, 2004.

REPORTABLE OUTCOMES

Lack A, Erikson KM, Dobson AW, Aschner M. Uranium uptake in rat brain endothelial cells and the possible link to divalent metal transporter-1 (DMT-1). Toxicol Sci 56, 2003 (abstract).

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